17P9

Metabolic control analysis of integrated energy metabolism in permeabilized neuroblastoma cells

I. Shevchuk, V. Chekulayev, N. Timohhina, K. Tepp, A. Klepinin, V. Saks, T. Kaambre
Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia
E-mail: igor@chemnet.ee

Today, there is only very limited information of the regulation of energy metabolism in neuroblastoma (NB) cells, although this is a prerequisite condition for development of more efficient treatment protocols. It is believed that NBs have increased rates of aerobic glycolysis and display a Warburg phenotype. The aim of the present study was to determine if there are specific alterations of aerobic energy metabolism in NBs, or if there is an overall down regulation of oxidative phosphorylation (OXPHOS) complexes. To clarify the mechanisms of regulation of mitochondrial respiration in NBs, we determined the apparent Km value for exogenously-added ADP. We found that the Km for ADP in non-differentiated and differentiated (normal) Neuro-2a (N2A) cells have very similar values, 20.3 ± 1.4 μM and 19.4 ± 3.2 μM, respectively, and the maximal (in the presence of 2 mM ADP) rate of O_2 consumption by differentiated N2A cells exceeds considerably (>2 times) that measured for non-differentiated cells. Our findings suggest that NB cells have, in comparison with normal differentiated neural cells, a decreased activity of OXPHOS.

Metabolic control analysis (MCA) performed on N2A cells suggest that in NBs the key sites of the regulation of OXPHOS are Complex-I (Flux control coefficient, FCC = 1.11), Complex-II (FCC = 0.99) and IV (FCC = 0.92), since FCC(s) for other mitochondrial complexes were found to be substantially lower and they have approximately equal values. In the mitochondria of differentiated N2A cells the key sites of respiratory regulation were found to be Complex-II (FCC = 1.34) and IV (FCC = 0.99). Moreover, our data suggest that in differentiated NB cells the Complex-II activity may exceed considerably that in malignant cells. It is most interesting that in N2A cells (like for example in breast cancer cells *in situ*) the sum of FCC(s) for ADP activated respiration exceeds significantly 1 normally observed in oxidative tissues and isolated mitochondria and is close to 4. This indicates the altered structure of the mitochondrial respiratory chain in N2A cells. Indeed, our results suggest that the mitochondrial respiratory chain and OXPHOS system contain large respiratory supercomplexes with direct substrate transfer inside these complexes.

doi:10.1016/j.bbabio.2012.06.357

17P10

The relative contribution of membrane potential and pH gradient regulates mitochondrial oxygen consumption at constant proton motive force

R. Springett, M.O. Ripple, N. Kim
Department of Radiology, Dartmouth Medical School, Hanover, NH 03755, USA
E-mail: RSpringett@Dartmouth.edu

Mitochondria are able to vary electron transport, oxygen consumption (VO_2) and ATP production to match cytosolic ATP demand. The primary intermediate of this coupling between ATP demand and VO_2 is thought to be the proton motive force (ΔP) however the heart literature reports large changes in the rate of ATP production with very little in ATP phosphorylation potential. Recently, we have developed a technique to quantify, in millivolts, the mitochondrial membrane potential ($\Delta\Psi$), pH gradient (ΔH^+) and hence ΔP in living cells from the oxidation state of the b-hemes of the bc_1 complex and cytochrome c (Cyt*c*) measured with multiwavelength cell spectroscopy (Kim et al., Biophys J, 2012. 102(7): p. 1194–1203). It was found that the addition of glutamine to cells doubles VO_2 at almost constant ΔP and this is achieved by increasing $\Delta\Psi$ and decreasing ΔH^+ resulting in a reduction of Cyt*c* and an increased driving force through cytochrome oxidase.

RAW 264.7 mouse macrophage cells were cultured overnight in glutamine-free RPMI media and then resuspended in the same media. Addition of 2 mM glutamine led to an increase in VO_2 from 21.6 ± 1.7 to 41.0 ± 3.0 μM/min (Mn ± SD, n = 6) over a period of 15 min. Prior to addition of glutamine, $\Delta\Psi$, ΔH^+ and ΔP were 133 ± 3, 52 ± 3 and 185 ± 3 mV, respectively, and 154 ± 3, 26 ± 3 and 180 ± 3 mV 15 min post glutamine. Thus VO_2 had doubled with only a 5 mV drop in ΔP. Analysis of the electron transport chain showed a small oxidation in NADH from -347 ± 4 to -336 ± 5 mV, an oxidation in the ubiquinone pool from 57 ± 4 to 73 ± 3 mV and a reduction in Cyt*c* from 301 ± 2 to 291 ± 3 mV.

Glutamine is imported into the matrix where it is converted to TCA cycle intermediates which are weak acids; their accumulation explains the acidification of the matrix. The bc_1 complex transduces $\Delta P + \Delta H^+$ millivolts of energy into the chemiosmotic gradient per electron. Because the bc_1 complex works close to equilibrium ($\Delta G = -6 \pm 1$ mV and -12 ± 1 mV before and after glutamine, respectively), Cyt*c* becomes more reduced with respect to the ubiquinone pool when ΔH^+ decreases. This 10 mV reduction in Cyt*c* provides an additional driving force for flux through cytochrome oxidase so that ΔP need only change by 5 mV for a doubling of flux.

The results have important implications for the regulation of the electron transport chain by mitochondrial ATP-sensitive potassium channels as high ATP will open the channel resulting in a decreased $\Delta\Psi$ and increased ΔH^+ thus switching off the electron transport chain.

doi:10.1016/j.bbabio.2012.06.358

17P11

Comparative investigation of bioenergetic properties of human colorectal and breast cancer

K. Tepp[1], T. Kaambre[1], V. Chekulayev[1], I. Shevchuk[1], M. Karu-Varikmaa[1], N. Timohhina[1], V. Valvere[2,3], V. Saks[1,4]
[1]*Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn, Estonia*
[2]*Oncology and Hematology Clinic at the North Estonia Medical Centre, Sütiste tee 19 Tallinn, Estonia*
[3]*Institute of Clinical Medicine, Technomedicum at Tallinn University of Technology,Akadeemia tee 15 Tallinn, Estonia*
[4]*INSERM U1055, Laboratory of Fundamental and Applied Bioenergetics, Joseph Fourier University, Grenoble, France*
E-mail: kersti.tepp@kbfi.ee

Theories of energy metabolism of cancer cells have been based on discovery by Otto Warburg of high glycolytic rate and increased levels of lactate even in the presence of oxygen, a phenomenon termed “aerobic glycolysis” or “Warburg effect”. This hypothesis has prevailed till the last decade, however, recent studies have shown that there are types of cancer cells with significant OXPHOS and in some malignant tissues the activity of respiratory chain is even higher than in normal cells of the same type.

In our study, to investigate the regulation of energy metabolism in cancer cells, the metabolic control analysis (MCA) has been applied to human breast (BC) and colorectal cancer (CC) permeabilized tissue samples in comparison with healthy tissue of same type. MCA helps